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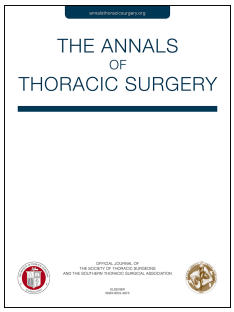

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HEPARIN BINDING PROTEIN IN ADULT HEART SURGERY

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Abstract

Background. Heparin binding protein (HBP) is released from neutrophilic secretory vesicles upon neutrophil adhesion on the endothelium. HBP mediates capillary hyperpermeability experimentally. In sepsis, HBP predicts organ dysfunction. Cardiopulmonary bypass induces both neutrophil activation and hyperpermeability. We hypothesized that in cardiopulmonary bypass, HBP is released in the reperfused coronary circulation concomitantly with neutrophil adhesion.

Methods. In 30 patients undergoing aortic valve replacement, concomitant blood samples were drawn from the coronary sinus and arterial line before aortic crossclamping and 5 min after reperfusion to calculate transcoronary differences. Plasma HBP concentrations, neutrophil markers lactoferrin and myeloperoxidase, myocardial injury marker heart-type fatty acid binding protein (hFABP) and leukocyte differential counts were measured.

Results. Arterial HBP was 4.1 (3.6–5.3) ng/ml preoperatively and 150.0 (108.2–188.6) ng/ml after aortic declamping. HBP increased 39-fold, lactoferrin 16-fold and myeloperoxidase 4-fold during cardiopulmonary bypass. Before cardiopulmonary bypass, there were marginal transcoronary differences in HBP [1.4 (-0.4–3.6) ng/ml, p=0.001] and hFABP [0.4 (-0.04–3.5) ng/ml, p=0.001] but not in the other parameters. During reperfusion, transcoronary HBP release [6.4 (1.8–13.7) ng/ml, p<0.001] was observed, concomitantly with transcoronary neutrophil sequestration [-0.14 (-0.28–0.01) x10^9/l, p=0.001] and transcoronary hFABP release [6.9 (3.0–25.8) ng/ml, p<0.001]. There were no transcoronary differences in lactoferrin or myeloperoxidase during reperfusion.

Conclusions. CPB results in substantial increase in circulating HBP. HBP is also released from the reperfused coronary circulation, concomitantly with coronary neutrophil adhesion and myocardial injury. HBP may be one candidate for a humoral factor mediating capillary leak in cardiopulmonary bypass.

Abstract word count: 248

Heparin binding protein (HBP) is a 37-kD granule protein of neutrophils. In neutrophils, it is located both in the secretory vesicles and the azurophilic granules [1]. This dual localization makes HBP an interesting biomarker for neutrophil activation. Because azurophilic granules show the lowest propensity to be exocytosed, HBP stored in these granules is mainly released in the tissues after extravasation. Secretory vesicles, on the contrary, are mobilized first [2]. This results in rapid increase in plasma HPB concentrations. As a sensitive indicator of intravascular neutrophil activation, HBP has gained increasing interest as a promising inflammatory biomarker during the recent years. High HBP is associated with severe sepsis and septic shock [3]. Importantly, HBP performs well in predicting meaningful clinical outcomes. Among septic patients, it predicts development of organ dysfunction in general [4] as well as more specifically circulatory failure [5, 6], respiratory failure [6, 7] and acute kidney injury [8, 9]. After cardiac arrest, HBP concentrations predict death [10].

Neutrophils are activated upon adhesion to endothelial cells. During firm adhesion, coupling of β2-integrins on neutrophil plasma membrane triggers release of HBP [11]. Intraluminally released HBP binds to glycosaminoglycans on the endothelium [12, 13]. HBP is proposed to have an important role in capillary leak. It induces endothelial hyperpermeability *in vitro* [11, 14]. In experimental conditions *in vivo*, intravenous administration of HBP induces acute lung injury with histological features similar to those after lipopolysaccharide administration [14]. Finally, in patients with septic shock, plasma HBP concentrations are associated with fluid overload and the degree of hypoxemia [14]. Like sepsis, also cardiopulmonary bypass (CPB) is associated with hyperpermeability [15, 16].

There is substantial amount of data demonstrating pathophysiological significance of activated neutrophils in experimental cardiac ischemia-reperfusion injury [17]. Although CPB is classically known to result in strong neutrophil activation, coronary neutrophil sequestration after aortic declamping has not been associated with concomitant transcoronary neutrophil activation in cardiac surgical patients [18]. Probably intracoronary neutrophil activation upon endothelial adhesion has been beyond the detection limit of available laboratory

methods. HBP has not been investigated in cardiac surgery. The hypothesis of the present study was two-fold. First, we hypothesized that CPB will induce more pronounced increase in HBP than in classical markers of neutrophil specific granules (lactoferrin, LF) and azurophilic granules (myeloperoxidase, MPO) [19]. Second, we hypothesized that HBP is sensitive enough to detect intracoronary neutrophil activation during reperfusion after aortic declamping in patients without coronary artery disease undergoing aortic valve reconstruction.

Patients and Methods

The study was approved by the Ethics Committee of Helsinki University Hospital. Written informed consent was obtained from all patients before the enrolment in the study. Thirty patients undergoing aortic valve replacement surgery (AVR) due to aortic valve stenosis were prospectively recruited. Exclusion criteria were as follows: coronary artery disease, left ventricular ejection fraction < 30%, systemic glucocorticoid medication or need for perioperative glucocorticoid substitution, immunosuppressive medication, other cardiac surgery than AVR in the same session, atrial fibrillation, insufficient cessation of antiplatelet and anticoagulation therapy (clopidogrel or ticagrelor < 5 days, low molecular weight heparins < 2 days). Anesthesia was induced with etomidate, alfentanil and rocuronium and maintained with sevoflurane, alfentanil infusion and rocuronium boluses. Pulmonary artery catheter was placed after the induction of anesthesia.

Affinity® NT (Medronic International Trading Sàrl, Tolochenaz, Switzerland) oxygenator and Trillium® (Medronic International Trading Sàrl, Tolochenaz, Switzerland) tubing were used. Patients received 300 IU/kg of heparin and additional boluses to achieve activated clotting time > 480 s. The aorta and right atrium were cannulated. The coronary sinus was cannulated with a 14Fr balloon tipped Retrograde Cardioplegia Catheter (Edwards Lifesciences, Irvine, CA, USA). The initial volume of the antegrade cold blood cardioplegia solution (4:1 cardioplegia solution to blood ratio) was double the volume needed for cessation of all cardiac electrical activity but never less than 1000 ml. Cardiac arrest was maintained by retrograde

infusion of 300 ml of blood cardioplegia solution (8:1 cardioplegia solution to blood ratio) every 20 minutes. Stöckert S5 roller pump (Sorin Group Deutschland Gmbh, Munich, Germany) was used for CPB, flow adjusted to 2.4 l/min x body surface area, mixed venous oxygen saturation maintained over 70%, fraction of inspired oxygen at 70%, partial pressure of carbon dioxide within 4.5 – 5-5 kPa and arterial pressure (MAP) at 40 – 60 mmHg. Patients were moderately cooled to 33-34 °C.

After weaning from CPB, protamine was administered 0.8-1 mg/100 IU of the initial heparin dose. After CPB, MAP of 60 – 80 mmHg was targeted. Hypotension after CPB was treated first by fluid loading (Ringers acetate, Albumin 4%) to achieve pulmonary capillary wedge pressure (PCWP) of 12 – 15 mmHg. Thereafter, norepinephrine infusion (0.01 – 0.1 µg/kg/min) was commenced if needed. After CPB, cardiac index (CI) of > 2.0 L/min/m2 was maintained by pre-load optimization (PCWP 12 – 15 mmHg) as well as epinephrine (0.02 – 0.2 µg/kg/min) and milrinone infusion (0.5 µg/kg/min) if needed. Hemoglobin target level was < 70g/L during CPB and < 80 g/L off-pump.

In addition to arterial samples, blood samples were obtained from the coronary sinus catheter. Correct placement of the catheter was verified with transesophageal ultrasound. In addition, comparisons of partial pressure of oxygen (pO2) and oxygen saturation (SO2) between simultaneously taken blood samples from the coronary sinus and pulmonary artery (i.e. the mixed venous sample) were made in parallel. Lower pO2 and lower SO2 in the coronary sinus than in the pulmonary artery was assumed to indicate correct placement of the coronary sinus cannula. Blood samples for research were drawn at four time points: (1) before induction of anesthesia, “preoperatively”; (2) immediately before ischemia, i.e. immediately before aortic cross clamping, “pre-ischemia”; (3) immediately before reperfusion, i.e. immediately before aortic declamping, “pre-reperfusion”; (4) 5 min after reperfusion, “reperfusion”. At all time points, 15 ml of arterial blood was drawn. At time point 1, the sample was taken from the peripheral arterial cannula. At time points 2 - 4, blood was drawn from the arterial line of the CPB. At time points 2 and 4, parallel blood samples of 15 ml were drawn from the coronary sinus into pyrogen free-syringes (BD Plastipak, Madrid, Spain). Samples

were immediately divided into two vacuum-tubes containing ethylenediaminetetraacetic acid (EDTA, BD Vacutainer, Plymouth, UK) and one tube containing sodium citrate (BD Vacutainer, Plymouth, UK). One EDTA tube was used for automated leucocyte differential count (Sysmex XE-2100, Sysmex Europe GmbH, Norderstedt, Germany). The rest of the tubes were transferred to ice-water bath and plasma was separated within 20 min by centrifugation in 2000 *g* at +4 °C. Plasma was stored in aliquots at -80 °C. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used for measurements of HBP (Axis-Shiled Diagnostics, Dundee, UK), LF (Hycult Biotech, Uden, The Netherlands), MPO (BioLegend, San Diego, Ca, USA) and heart-type fatty acid binding protein (hFABP, Hycult Biotech, Uden, The Netherlands). Since hFABP is a rapid and sensitive biomarker of cardiomyocyte injury, it was used as a positive control biomarker for detection of transcoronary plasma concentration differences of a parameter [20]. Plasma aliquots that were not thawed previously were used for ELISA measurements. Measurements of LF were originally conducted for another so far unpublished study and transcoronary difference of LF was not available at "pre-ischemia" time point. In addition to ELISA analyses, troponin T (TnT) was measured at the clinical laboratory of the hospital exactly at 22 hours and 48 hours after aortic declamping.

Data were analyzed with SPSS 25 for Windows. The study was observational by nature. As there was no intervention, power analysis for the size of a treatment group was not applicable. Non-parametric approach was used due to small patient number. Friedman's test was used for testing differences as a function of time. Post-hoc, differences between every pair of two consecutive time points (preoperative vs. pre-ischemia; pre-ischemia vs. pre-reperfusion; pre-reperfusion vs. post-reperfusion) were tested using Wilcoxon signed rank test with Bonferroni correction for three comparisons. Likewise, comparison of fold-increase between HBP, LF and MPO at a same time point was undertaken with Friedman's test and Wilcoxon signed rank test as a post-hoc test with Bonferroni correction for three comparisons (HBP vs. LF; HBP vs. MPO; LF vs. MPO). Wilcoxon signed rank test was also used for comparison of transcoronary differences. Spearman's test was used for bivariate correlations. After Bonferroni correction due to three comparisons, p-values < 0.017 were considered statistically significant. Otherwise, p-values < 0.05 were considered significant. Data are expressed as median and interquartile range (IQR) or depicted as box-plots.

Results

The study group consisted of fifteen males and fifteen females. The age of the patients was 66 (61-73) years, the CPB time 101 (85-114) min and aortic crossclamping time 70 (58-79) min.

Verification of the placement of the coronary sinus catheter

In the verification of correct placement of the coronary sinus catheter, pO2 and SO2 were higher in the coronary sinus sample than in the simultaneously taken mixed venous sample in one patient before CPB (the sample was deleted from all analyses). The catheter was readjusted and following samples proved valid pO2 and SO2 measurements. In all other patients, pO2 and SO2 were lower in the coronary sinus sample than in the simultaneously taken mixed venous sample both before CPB and after reperfusion (data not shown). As a positive technical control biomarker for the measurement of transcoronary plasma concentration differences, hFABP was significantly higher in the coronary sinus samples than in the simultaneously taken arterial samples both before CPB [artery: 9.8 (6.0-17.7) ng/ml vs. coronary sinus: 11.2 (6.2-18.3) ng/ml; difference: 0.4 (-0.04-3.5) ng/ml, p=0.002] and after reperfusion [artery: 36.7 (23.9-59.2) ng/ml vs. coronary sinus: 46.1 (28.8-90.5) ng/ml; difference: 6.9 (3.0-25.8) ng/ml, p<0.001].

Changes in the arterial samples as a function of time

Plasma concentrations of HBP, LF and MPO as well as neutrophil count in arterial samples as a function of time are presented in the Table 1. All these parameters increased significantly during the study period (p<0.001 for all parameters). In HBP, LF and MPO concentrations as well as in neutrophil count, there was a significant difference (all p<0.01) in every pair of two consecutive time points (preoperative vs. pre-ischemia; pre-ischemia vs. pre-reperfusion; pre-reperfusion vs. post-reperfusion). There were significant increases also in monocyte and lymphocyte counts in arterial samples during the study period (data not shown). For relative comparison between HBP, LF and MPO, granule protein levels at different time points are presented as fold-increase, i.e. as multiplies of respective baseline values in the Figure 1. During the operation, HBP increased 39-fold, LF 16-fold and MPO 4-fold, compared with the preoperative level.

Changes across the coronary circulation

Before CPB, HBP was marginally but statistically significantly higher in the coronary sinus than in simultaneously taken arterial sample (Fig. 2). Transcoronary gradients of neither neutrophil (Fig. 2), monocyte (data not shown) nor lymphocyte counts (data not shown) nor MPO concentrations (Fig. 2) were observed before CPB. After reperfusion, neutrophil count was significantly lower in the coronary sinus than the arterial sample, indicating a transcoronary neutrophil sequestration of -0.14 (-0.28 – 0.01) x10^9/l (p=0.001, Fig. 2). After reperfusion, there were also statistically significant entrapment of both monocytes [transcoronary difference: -0.06 (-0.11 – 0.02) x10^9/l, p=0.013] and lymphocytes [transcoronary difference: -0.020(-0.065 – -0.005) x10^9/l, p=0.021]. After reperfusion, a positive transcoronary HBP concentration gradient was observed, indicating transcoronary release of HBP. This transcoronary HBP concentration difference did not correlate with transcoronary neutrophil difference, ischemia time or TnT either at 22 hours or 48 hours after cardiac reperfusion. Transcoronary concentration gradients of neither LF nor MPO were observed before CPB or after reperfusion (Fig. 2).

Comment

The present results have a three-fold message. First, a substantial increase in plasma concentrations of HBP occurs during CPB. Second, in addition to systemic increase, HBP is also released locally in the reperfused coronary circulation, concomitantly with coronary neutrophil adhesion. Third, as a biomarker of intravascular neutrophil activation, HBP is superior to granule proteins of either specific granules (LF) or azurophilic granules (MPO).

The median level of HBP is approximately 30 ng/ml in septic patients without organ dysfunction and approximately 50 ng/ml in patients with organ dysfunction. [3, 5]. In our patients undergoing cardiac surgery, a median HBP level of 41 ng/ml was reached already before CPB and a median level of 150 ng/ml

was observed after reperfusion. In septic patients, plasma HBP concentration higher than 15 ng/ml predicts development of organ dysfunction and is associated with four-fold increase in the risk of death [3]. As clinical scenario of sepsis is different from that of CPB, direct comparison of septic and cardiac surgical patients cannot be done. Still, it can be concluded that substantially high levels of HBP were observed during CPB. HBP induces vascular leak in experimental conditions [11, 14]. In septic patients, HBP is correlated with the degree of fluid overload and hypoxemia [14]. Plasma obtained from CPB patients mediates endothelial hyperpermeability *in vitro* [16]. Clinical significance of HBP cannot be judged in the present observational study of small patient number. Nevertheless, it is tempting to speculate that HBP may be one candidate for a humoral mediator of hyperpermeability during CPB [15, 16].

Numerous experimental studies have shown the pathophysiological significance of activated neutrophils in experimental cardiac ischemia-reperfusion injury [17]. Although intracoronary entrapment of neutrophils during reperfusion in cardiac surgery has been observed long ago, conclusive evidence of transcoronary neutrophil activation has been difficult to obtain [18, 21]. Since also lymphocytes and monocytes are sequestrated in the coronary circulation, one may ask if coronary leukocyte entrapment in clinical heart surgery is only a passive phenomenon. During cardiac reperfusion at 5 minutes after aortic declamping, we observed statistically highly significant release of HBP in the coronary circulation. Median transcoronary HBP concentration difference of 6.6 ng/ml was observed and the highest measured difference was 51.6 ng/ml. As we took simultaneous samples of blood entering and leaving the coronary circulation, the transcoronary concentration difference measured momentous production of HBP. The cumulative production was likely even more excessive. Furthermore, as HBP is bound to the endothelial glycocalyx, observed transcoronary concentration difference probably reflects only a spillover of all HBP released from the adhered neutrophils. Taken together, compared with systemic HBP levels in cardiac surgical patients and especially in septic patients, intracoronary production of HBP can be regarded strong [3, 5]. Two conclusions can be drawn. First, intravascular neutrophil activation in the coronary circulation indeed occurs during cardiac reperfusion in clinical heart surgery. Second, this neutrophil activation results in high local concentration of HBP in the coronary vascular bed. Again, it can be speculated that accumulating HBP may

have significance in coronary endothelial cell injury and myocardial edema formation, occurring both in experimental and clinical cardiac surgery [22, 23]. Of note, coronary HPB release was accompanied with the coronary release of hFABP that is an early and sensitive biomarker of myocardial injury [20].

Compared with the preoperative level, HBP increased up to 39-fold after aortic declamping. The corresponding increases in LF was 16-fold and that in MPO only 4-fold. Approximately the same proportional differences in plasma concentrations of the three granule proteins were observed already at the beginning of CPB, i.e. at a moderate level of neutrophil activation. Furthermore, although clear coronary release of HBP was observed after cardiac reperfusion, both LF and MPO failed to detect intracoronary neutrophil activation. Of note, a marginal but statistically significant transcoronary increase of HBP was observed even immediately before aortic crossclamping. Heparinization and initiation of CPB probably result in increased circulating HBP concentrations at this early phase of surgery. However, concomitant marginal release of also hFABP was observed. Thus, these two preischemic findings may also reflect subtle myocardial and endothelial injury due to cannulation and other surgical manipulation of the heart before CPB. These findings further underscore the sensitivity of HBP as a biomarker. The differences between neutrophil granule protein concentrations reflect the timing and sequence of neutrophil degranulation during physiological neutrophil functions. β2-integrin signaling triggers the release of HBP-containing secretory vesicles upon neutrophil adhesion to the endothelium, i.e. in the intravascular space [11]. Azurophilic granules (MPO), on the other hand, are important in the formation of a phagosome for killing of microbes in the tissues [2]. Specific granules (LF) are somewhere in between. They take part in phagosome formation but they also contain β2-integrins that are needed in endothelial adhesion [2]. The fact that secretory vesicles are meant to be released intravascularly probably makes HBP a superior neutrophil biomarker in plasma. Neutrophilic granule proteins of only either specific or azurophilic granules have been measured in cardiac surgery in the past. No clinical impact has been reached in these studies. In future studies, HBP as a biomarker may offer a different picture of the clinical significance of neutrophil activation in cardiac surgery.

The chosen patient population is both a strength and a weakness of the study. Our focus was on the coronary reperfusion phenomenon and thus coronary sinus blood samples were obtained. We wanted to avoid the confounding effect of coronary artery disease. This reduced, not only the number of patients suitable for the study, but also ischemia times and thus variability of ischemic challenge. A small and homogenous patient population and fairly subtle ischemic insult may explain the fact that we did not find correlations between HBP and clinical indices, i.e. troponin or aortic crossclamping time. On the other hand, both the pre- and postischemic coronary HBP release followed the pattern of the coronary hFABP release. While substantial coronary release of HBP was observed in patients undergoing isolated AVR, it is likely that coronary neutrophil activation would have been even stronger in patients with concomitant coronary pathology and longer ischemia times. Thus, the present patient population serves as a meaningful verification of reperfusion-induced coronary neutrophil activation in clinical heart surgery. Correct placement of the coronary sinus catheter was rigorously verified with multiple methods, i.e. transesophageal cardiac ultrasound as well as measuring pO2, SO2 and hFABP. Furthermore, artifact neutrophil activation *in vitro* was reduced to minimum, when blood samples for separation of plasma were immediately cooled on an ice-water bath and centrifuged at + 4 °C and plasma separated within 20 min.

In conclusion, CPB induced a substantial increase in HBP plasma concentrations in the systemic circulation. Furthermore, HBP was also released in the reperfused coronary circulation at the time of coronary neutrophil adhesion and myocardial injury. Both experimental *in vitro* and *in vivo* evidence has accumulated for the key role of HBP in the pathophysiology of capillary leak [11, 14]. According to the present results, HBP is one candidate for a humoral factor mediating capillary leak in CPB [16]. In addition, acute kidney injury is a frequent complication of CPB. HBP predicts acute kidney injury in septic patients [8, 9]. HBP may perform well as a biomarker of organ dysfunction also in cardiac surgery. The clinical significance of HBP needs to be verified in a larger patient cohort.

References

1. Fisher J, Linder A. Heparin-binding protein: a key player in the pathophysiology of organ dysfunction in sepsis. J Intern Med 2017;281:562-74.
2. Tapper H, Karlsson A, Mörgelin M, Flodgaard H, Herwald H. Secretion of heparin-binding protein from human neutrophils is determined by its localization in azurophilic granules and secretory vesicles. Blood 2002;99:1785-93.
3. Linder A, Åkesson P, Inghammar M, Treutiger CJ, Linnér A, Sundén-Cullberg J. Elevated plasma levels of heparin-binding protein in intensive care unit patients with severe sepsis and septic shock. Crit Care 2012;16:R90.
4. Linder A, Arnold R, Boyd JH, et al. Heparin-binding protein measurement improves the prediction of severe infection with organ dysfunction in the emergency department. Crit Care Med 2015;43:2378-86.
5. Linder A, Christensson B, Herwald H, Björck L, Akesson P. Heparin-binding protein: an early marker of circulatory failure in sepsis. Clin Infect Dis 2009;49:1044-50.
6. Tydén J, Herwald H, Sjöberg F, Johansson J. Increased plasma levels of heparin-binding protein on admission to intensive care are associated with respiratory and circulatory failure. PLoS One 2016;11:e0152035.
7. Lin Q, Shen J, Shen L, Zhang Z, Fu F. Increased plasma levels of heparin-binding protein in patients with acute respiratory distress syndrome. Crit Care 2013;17:R155.
8. Fisher J, Russell JA, Bentzer P, et al. Heparin-Binding Protein (HBP): A causative marker and potential target for heparin treatment of human sepsis-induced acute kidney injury. Shock 2017;48:313-20.
9. Tydén J, Herwald H, Hultin M, Walldén J, Johansson J. Heparin-binding protein as a biomarker of acute kidney injury in critical illness. Acta Anaesthesiol Scand 2017;61:797-803.
10. Ristagno G, Masson S, Tiainen M, et al.; FINNRESUSCI Study Group. Elevated plasma heparin-binding protein is associated with early death after resuscitation from cardiac arrest. Crit Care 2016;20:251.

11. Gautam N, Olofsson AM, Herwald H, et al. Heparin-binding protein (HBP/CAP37): a missing link in neutrophil-evoked alteration of vascular permeability. Nat Med 2001;7:1123-7.
12. Olofsson AM, Vestberg M, Herwald H, et al. Heparin-binding protein targeted to mitochondrial compartments protects endothelial cells from apoptosis. J Clin Invest 1999;104:885-94.
13. Soehnlein O, Xie X, Ulbrich H, et al. Neutrophil-derived heparin-binding protein (HBP/CAP37) deposited on endothelium enhances monocyte arrest under flow conditions. J Immunol 2005;174:6399-405.
14. Bentzer P, Fisher J, Kong HJ, et al. Heparin-binding protein is important for vascular leak in sepsis. Intensive Care Med Exp 2016;4:33.
15. Lundemoen S, Kvalheim VL, Mongstad A, Andersen KS, Grong K, Husby P. Microvascular fluid exchange during pulsatile cardiopulmonary bypass perfusion with the combined use of a nonpulsatile pump and intra-aortic balloon pump. J Thorac Cardiovasc Surg 2013;146:1275-82.
16. Koning NJ, Overmars MA, van den Brom CE, et al. Endothelial hyperpermeability after cardiac surgery with cardiopulmonary bypass as assessed using an in vitro bioassay for endothelial barrier function. Br J Anaesth 2016;116:223-32.
17. Ferrari R, Biscaglia S, Malagù M, Bertini M, Campo G. Can we improve myocardial protection during ischaemic injury? Cardiology 2016;135:14-26.
18. Pesonen EJ, Vento AE, Rämo J, Vuorte J, Jansson SE, Repo H. Nitecapone reduces cardiac neutrophil accumulation in clinical open heart surgery. Anesthesiology 1999;91:355-61.
19. Pesonen EJ, Korpela R, Peltola K, et al. Regional generation of free oxygen radicals during cardiopulmonary bypass in children. J Thorac Cardiovasc Surg 1995;110:768-73.
20. Liou K, Ho S, Ooi SY. Heart-type fatty acid binding protein in early diagnosis of myocardial infarction in the era of high-sensitivity troponin: a systematic review and meta-analysis. Ann Clin Biochem 2015;52:370-81.
21. Semb AG, Forsdahl K, Vaage J. Granulocyte and eicosanoid gradients across the coronary circulation during myocardial reperfusion in cardiac surgery. Eur J Cardiothorac Surg 1990;4:543-8.

22. Yeh CH, Wang YC, Wu YC, Chu JJ, Lin PJ. Continuous tepid blood cardioplegia can preserve coronary endothelium and ameliorate the occurrence of cardiomyocyte apoptosis. Chest 2003;123:1647-54.

23. Cuccurullo L, Accardo M, Agozzino L, Blasi F, Esposito S, Vosa C. Ultrastructural pathology of pediatric myocardium in acute ischemia: bioptic study before and after treatment with cardioplegic solution. Ultrastruct Pathol 2006;30:453-60.

Table 1. Arterial values of the parameter during the study period.

	preop	**pre-ischemia**	**pre-reperfusion**	**post-reperfusion**
HBP, ng/ml	4.1 (3.6-5.3)	41.0 (34.4.-54.9)	132.7(96.1-198.9)	150.0 (108.2-188.6)
LF, ng/ml	61.7 (48.4-132.4)	402.8 (310.6-541.1)	975.0 (598.0-1275.3)	1176.5 (653.2-1377.0)
MPO, ng/ml	58.5 (42.4-75.5)	231.3 (142.2-257.2)	372.1 (273.9-426.2)	411.2 (287.2-465.8)
neutr, x10^9/l	4.1 (3.1-4.6)	2.4 (1.5-3.6)	5.5 (3.6-8.9)	6.0 (4.4-8.7)

HBP, heparin binding protein; IQR, interquartile range; LF, lactoferrin; MPO, myeloperoxidase; neutr, neutrophil count. All parameters increased significantly during the study period (all $p<0.001$).

Figure legends

Figure 1. Plasma levels of heparin binding protein (HBP, dark grey bars), lactoferrin (LF, light grey bars) and myeloperoxidase (MPO, white bars) in arterial samples during the study period. The levels are presented as fold-increase, i.e. as multiplies of respective baseline values. All parameters increased significantly (all $p<0.001$). HBP, LF and MPO all differed from each other before CPB, before reperfusion and after reperfusion (all $p<0.001$).

Figure 2. Transcoronary differences (i.e. the difference between coronary sinus and artery) of neutrophil count as well as plasma concentrations of heparin binding protein (HBP), lactoferrin (LF, pre-ischemia missing) and myeloperoxidase (MPO) before CPB and 5 min after aortic declamping. **, $p<0.01$, coronary sinus vs. artery; ***, $p<0.001$, coronary sinus vs. artery.

List of abbreviations

AVR	aortic valve replacement
CI	cardiac index
CPB	cardiopulmonary bypass
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
HBP	heparin binding protein
hFABP	heart-type fatty acid binding protein
IQR	interquartile range
LF	lactoferrin
MAP	mean arterial pressure
MPO	myeloperoxidase
PCWP	pulmonary capillary wedge pressure
pO2	partial pressure of oxygen
SO2	oxygen saturation
TnT	troponin T